Iron *bis*-glycine chelate competes for the nonheme-iron absorption pathway[1–3]

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ABSTRACT

Background: The enterocytic absorption pathway of the food fortificant iron *bis*-glycine chelate has been the subject of controversy because it is not clear whether that substance uses the classic nonheme-iron absorption pathway or a pathway similar to that of heme absorption.

Objective: The objective was to study the absorption pathway of iron *bis*-glycine chelate in human subjects.

Design: Eighty-five healthy adult women were selected to participate in 1 of 6 iron-absorption studies. Study A involved the measurement of the dose-response curve of the absorption of ferrous sulfate (through a nonheme-iron absorption pathway); study B involved the competition of iron *bis*-glycine chelate with ferrous sulfate for the nonheme-iron absorption pathway; study C involved the measurement of the dose-response curve of heme-iron absorption; study D involved the competition of iron *bis*-glycine chelate with hemoglobin for the heme-iron absorption pathway; and studies E and F were the same as studies A and B, except that the iron *bis*-glycine chelate was encapsulated in enteric gelatin capsules so that it would not be processed in the stomach.

Results: Iron from the *bis*-glycine chelate competed with ferrous sulfate for the nonheme-iron absorption pathway. Iron from the *bis*-glycine chelate also competed with ferrous sulfate for absorption when liberated directly into the intestinal lumen. Iron from the *bis*-glycine chelate did not compete with heme iron for the heme-iron absorption pathway.

Conclusion: The iron from iron *bis*-glycine chelate delivered at the level of the stomach or duodenum becomes part of the nonheme-iron pool and is absorbed as such.   *Am J Clin Nutr* 2002;76:577–81.

INTRODUCTION

The prevalence of iron deficiency in industrialized countries is < 6%. In contrast, in the developing world, it is a serious problem, principally affecting infants, children, and women (1, 2). Iron fortification of food is the most widely recommended strategy for preventing iron deficiency (3). The steps required to establish an iron-fortification program should be followed carefully (4, 5). Of these, one of the most difficult is the selection of an appropriate iron compound. Possible foods that could be consumed as vehicles for iron in developing countries contain phytates and polyphenols that inhibit nonheme-iron absorption. Soluble compounds, despite their low cost and high iron bioavailability, could induce organoleptic changes in the food vehicle. In comparison, insoluble compounds are more stable and do not create adverse effects in foods, but they have a lower rate of absorption (5).

Recently, several studies showed that iron *bis*-glycine chelate has better iron bioavailability than does ferrous sulfate, especially when the vehicles of consumption are foods that inhibit nonheme-iron absorption (6–8). This chelate is composed of 2 glycine molecules bound to a ferrous cation to form a double heterocyclic ring compound. It has been proposed that this configuration protects the iron from dietary inhibitors and intestinal interactions, which explains its high bioavailability. The absorption pathway of iron derived from the *bis*-glycine chelate is unknown, but it has been suggested that its absorption route is different from the routes of heme and nonheme iron (9). Nevertheless, recent evidence supports the notion that iron from the *bis*-glycine chelate could enter the common nonheme-iron pool: the bioavailability of iron from the *bis*-glycine chelate is enhanced by ascorbic acid (6), and the absorption of amino-chelated iron correlated inversely with body iron stores (6, 7, 10). Both results would be expected if the iron from the *bis*-glycine chelate is absorbed through the nonheme-iron absorption pathway. The aim of the present study was to further elucidate the absorption pathway of the iron *bis*-glycine chelate through the comparative analysis of its effect on heme- and nonheme-iron absorption.

SUBJECTS AND METHODS

Study design

Six studies were performed to examine the competitive effect of nonlabeled heme or nonheme iron, ferrous sulfate, and iron *bis*-glycine chelate on the absorption of labeled ferrous sulfate or hemoglobin. The goals of each study were as follows. Study A was intended to measure the dose-response curve of nonheme-iron absorption (ferrous sulfate), study B was intended to examine the competition between iron *bis*-glycine chelate and

ferrous sulfate for the nonheme-iron absorption pathway, study C was intended to measure the dose-response curve of heme-iron absorption (hemoglobin), and study D was intended to examine the competition between iron *bis*-glycine chelate and hemoglobin for the heme-iron absorption pathway. Study E was intended to investigate the dose-response curve of nonheme-iron absorption when the ferrous sulfate was given in an enteric capsule, and study F was intended to observe the competition between iron *bis*-glycine chelate and ferrous sulfate, both given in enteric capsules covered with the same polymer, for the nonheme-iron absorption pathway. Because the iron *bis*-glycine chelate molecules may dissociate at low pH, studies A and B were designed to examine the behavior of the iron *bis*-glycine chelate that was liberated directly at the stomach level, whereas studies E and F were designed to examine the behavior of the iron *bis*-glycine chelate that was liberated directly at the intestinal level.

Subjects

Eighty-five healthy women between 35 and 45 y of age were selected and randomly assigned to 1 of 6 iron-absorption studies (<14 subjects/study). None of the subjects were pregnant (confirmed by a negative test for human chorionic gonadotropin in urine), and each was using an intrauterine device as her method of contraception at the time of the study.

Written, informed consent was obtained from all the volunteers before the studies began. The protocol was approved by the Ethics Committee of the Institute of Nutrition and Food Technology, and the doses of radioactive isotopes used were approved by the Chilean Commission on Nuclear Energy.

Isotopic studies

Iron isotopes (^{59}Fe and ^{55}Fe) of high specific activity were used as tracers (NEN Life Science Products, Inc, Boston). The doses of the compounds labeled with the iron isotopes were given to the subjects in number 0 gelatin capsules (Reutter Co, Santiago, Chile) or gelatin capsules covered with an enteric polymer (Eudragit L30 D55; Röhm GmbH, Darmstadt, Germany).

Labeled hemoglobin was prepared by using red blood cells from rabbits. New Zealand rabbits weighing <3 kg received an intravenous injection of 74 MBq ^{55}Fe or 37 MBq ^{59}Fe as ferric citrate (NEN Life Science Products, Inc) diluted in 10 mL of a solution containing 9 g sodium chloride/L. Fifteen days later, the animals' blood was drained through a cardiac puncture. The radioactive red blood cells were centrifuged and washed in a saline solution, hemolyzed by freezing, and dehydrated by lyophilization. Labeled hemoglobin with a specific activity of 475 kBq ^{59}Fe and 2460 kBq ^{55}Fe per mg of iron was obtained. The labeled hemoglobin was mixed in dry form with untagged bovine hemoglobin (11) such that the result was a dose of 37 kBq ^{59}Fe or 111 kBq ^{55}Fe per 0.5 mg elemental iron. Finally, these compounds were packaged in gelatin capsules.

In all 6 studies, graded iron doses were administered on days 1, 2, 15, and 20, with ^{55}Fe being given on days 1 and 15 and ^{59}Fe being given on days 2 and 20. The labeled compounds were administered in one capsule containing 0.5 mg Fe as ferrous sulfate or hemoglobin labeled with ^{55}Fe or ^{59}Fe. At the same time, graded doses of unlabeled iron were administered in ≥1 capsule. The doses were administered after a nocturnal fast, and subjects were not allowed to eat again until 4 h after ingestion of the doses.

In studies A and E, the subjects received 0.5 mg Fe as ferrous sulfate labeled with 111 kBq ^{55}Fe or 37 kBq ^{59}Fe together with 0, 4.5, 49.5, and 99.5 mg Fe as ferrous sulfate. In studies B and F, the subjects received 0.5 mg Fe as ferrous sulfate labeled with 111 kBq ^{55}Fe or 37 kBq ^{59}Fe together with 0, 4.5, 49.5, and 99.5 mg Fe as *bis*-glycine chelate. In studies C and D, the subjects received 0.5 mg Fe as hemoglobin labeled with ^{55}Fe or ^{59}Fe together with 0, 2.5, 14.5, and 29.5 mg Fe as hemoglobin or *bis*-glycine chelate, respectively. The graded iron concentrations provided for studies C and D were lower than those in the other studies because of the difficulties in providing comparable amounts of iron as hemoglobin (30 mg heme iron is equivalent to 10 g dehydrated blood). Venous blood samples were obtained on days 15 and 35 to measure the circulating radioactivity and to determine the iron status of the subject. Hemoglobin, free erythrocyte protoporphyrin, serum iron, total-iron-binding capacity, transferrin saturation, and serum ferritin were measured from these samples (12). To evaluate the iron status of the women, we used 120 g/L as the lower-normal limit for hemoglobin, 15% for transferrin saturation, and 12 mg/L for serum ferritin; we used 1.42 mmol/L red blood cells as an upper-normal limit for free erythrocyte protoporphyrin. Depleted iron stores were defined as below-normal serum ferritin concentrations, iron deficiency without anemia was defined as normal hemoglobin and ≥2 abnormal laboratory results, and iron deficiency anemia was defined as below-normal hemoglobin and ≥2 abnormal laboratory results.

For the calculation of total radioactivity ingested, the radioactivity of 6 aliquots of each compound was counted and these values were used as standards. Measurement of blood radioactivity was performed in duplicate venous samples according to the technique of Eakins and Brown (13). The samples were counted in sufficient time to obtain a counting error of <3% in a liquid scintillation counter (LS 5000 TD; Beckman Instruments, Fullerton, CA). The percentages of absorption were calculated on the basis of blood volumes estimated for height and weight (14) and assuming an 80% incorporation of the radioisotope into the erythrocyte (15). This method is reproducible in our laboratory with a CV of 5%.

Statistics

For the purposes of comparison, absorption of the 0.5 mg dose of iron as ferrous sulfate or hemoglobin (ie, the reference dose) was defined as 100% absorption. Because the percentages of iron absorption and serum ferritin concentrations had a skewed distribution, these values were converted to logarithms before we calculated means and SDs or performed statistical analyses. The results were retransformed into anti-logarithms to recover the original units and were expressed as geometric means ± SDs in the tables and as geometric means ± SEMs in the figures (16). The least-squares mean was used to fit individual data to the nonlinear dose-response curves. Dose-response curves were compared with each other by using the general linear models procedure (SAS ONLINEDOC 8.0; SAS Institute Inc, Cary, NC).

RESULTS

The iron nutritional status of most of the subjects who participated in these studies was normal. Only 4 of 85 women presented with iron deficiency anemia, 6 had iron deficiency without anemia, and 7 had iron depletion. No significant differences in the iron nutrition status values were found between the groups. We

TABLE 1
Percentage of absorption of 0.5 mg Fe radioisotope as ferrous sulfate or hemoglobin in subjects receiving graded doses of iron as ferrous sulfate, iron *bis*-glycine chelate, or hemoglobin[1]

|	Study					
	A	B	C	D	E	F
$^{59}FeSO_4$	43.2 (20.7–89.9)	40.7 (15.8–105.0)	—	—	50.1 (28.9–86.9)	58.1 (35.5–94.9)
$^{55}FeSO_4$ + 4.5 mg $FeSO_4$ or *bis*-glycine	24.1 (10.1–57.5)	17.9 (6.2–51.7)	—	—	28.6 (16.9–48.5)	36.0 (19.3–67.3)
$^{59}FeSO_4$ + 49.5 mg $FeSO_4$ or *bis*-glycine	13.4 (7.0–25.6)	14.1 (7.3–27.5)	—	—	17.5 (8.2–37.4)	10.6 (6.4–17.5)
$^{55}FeSO_4$ + 99.5 mg $FeSO_4$ or *bis*-glycine	13.0 (8.3–20.2)	15.3 (9.9–23.7)	—	—	14.4 (7.3–28.2)	6.6 (3.4–12.8)
^{59}Fe-Hb	—	—	27.1 (13.7–53.3)	27.4 (13.5–55.3)	—	—
^{55}Fe-Hb + 2.5 mg Fe-Hb or *bis*-glycine	—	—	13.2 (8.4–20.8)	20.4 (9.6–43.5)	—	—
^{59}Fe-Hb + 14.5 mg Fe-Hb or *bis*-glycine	—	—	14.6 (10.4–20.4)	19.2 (9.5–38.8)	—	—
^{55}Fe-Hb + 29.5 mg Fe-Hb or *bis*-glycine	—	—	7.4 (2.7–20.3)	20.6 (11.8–35.8)	—	—

[1] Geometric $\bar{x}$; range in parentheses. Hb, hemoglobin. Study A: dose-response values of nonheme-iron absorption (ferrous sulfate); study B: competition between iron *bis*-glycine chelate and ferrous sulfate; study C: dose-response values of heme-iron absorption (Hb); study D: competition between iron *bis*-glycine chelate and Hb; studies E and F: dose-response values of nonheme-iron absorption when the ferrous sulfate (E) and iron *bis*-glycine chelate (F) were given in an enteric capsule.

found a significant direct correlation between free erythrocyte protoporphyrin and iron absorption ($r = 0.63$) and a significant inverse correlation between serum ferritin and iron absorption ($r = -0.54$).
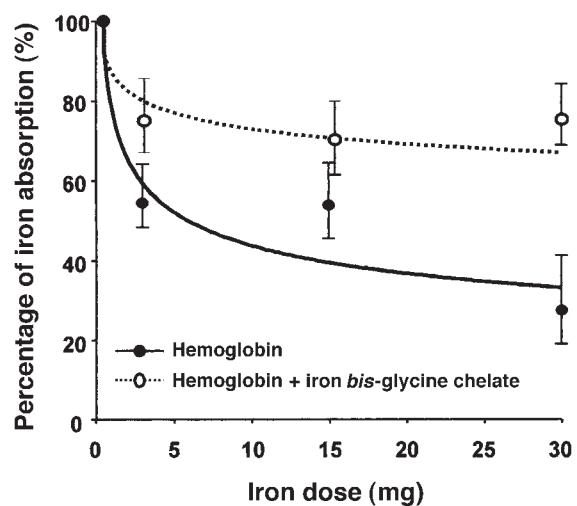
Geometric mean percentages of absorption in the 6 studies are shown in **Table 1**. According to the dose-response curves of corrected values for nonheme-iron absorption, 100.0%, 55.8%, 31.0%, and 30.1% of the doses of 0.5, 5, 50, and 100 mg Fe as ferrous sulfate, respectively (study A), were absorbed, whereas, when iron *bis*-glycine chelate competed with ferrous sulfate for the nonheme-iron absorption pathway (study B), 100.0%, 44.1%, 34.7%, and 37.7% of the 0.5-, 5-, 50-, and 100-mg Fe doses, respectively, were absorbed. The fitted dose-response curves were not significantly different [repeated-measures analysis of variance (ANOVA)], as shown in **Figure 1**.

The significant differences in the fitted dose-response curves between hemoglobin (study C) and *bis*-glycine chelate (study D) when repeated-measures ANOVA was applied ($P < 0.001$) are shown in **Figure 2**. The dose-response curves showed that 100.0%, 74.8%, 70.1%, and 75.2% of the doses of 0.5, 3, 15, and 30 mg Fe, respectively, were absorbed in study D, whereas 100.0%, 48.7%, 53.9%, and 27.3% of the doses of 0.5, 3, 15, and 30 mg Fe, respectively, were absorbed in study C.
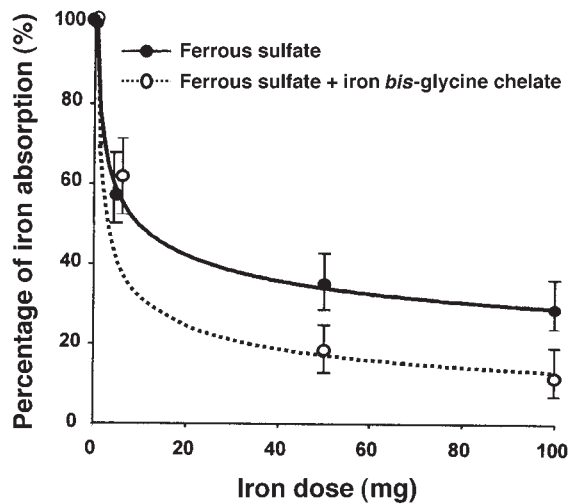
The dose-response curve of ferrous sulfate liberated in the intestinal lumen showed corrected values of iron absorption of 100.0%, 57.1%, 34.9%, and 28.7% for 0.5, 5, 50, and 100 mg Fe as ferrous sulfate, respectively (study E), whereas, when iron *bis*-glycine chelate liberated at the intestinal level competed with ferrous sulfate for the nonheme-iron absorption pathway (study F),
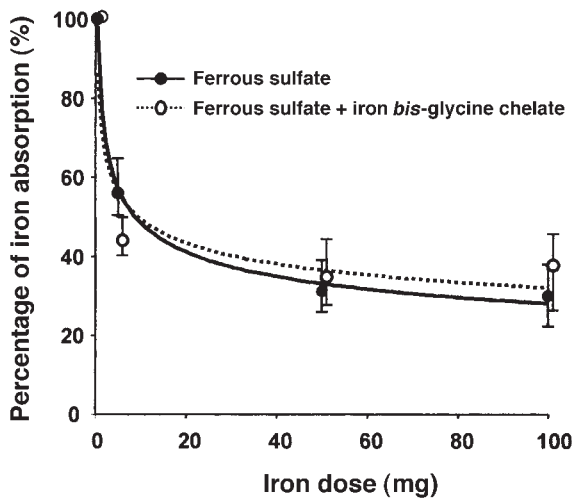
FIGURE 1. Fitted dose-response curves of corrected geometric mean ($\pm$ SEM) percentages of absorption of ferrous sulfate alone and of ferrous sulfate in competition with iron *bis*-glycine chelate through the nonheme-iron absorption pathway. The curves were not significantly different (repeated-measures ANOVA) and indicate that iron *bis*-glycine chelate competed with ferrous sulfate for the nonheme-iron absorption pathway. Absorption of the 0.5-mg dose of iron as ferrous sulfate or hemoglobin (ie, the reference dose) is defined as 100% absorption.

FIGURE 2. Fitted dose-response curves of corrected geometric mean ($\pm$ SEM) percentages of absorption of hemoglobin alone and of hemoglobin in competition with iron bis-glycine chelate through the heme-iron absorption pathway. The curves were significantly different ($P < 0.01$, repeated-measures ANOVA) and indicate that iron *bis*-glycine chelate did not compete with hemoglobin for the heme-iron absorption pathway. Absorption of the 0.5-mg dose of iron as ferrous sulfate or hemoglobin (ie, the reference dose) is defined as 100% absorption.

FIGURE 3. Fitted dose-response curves of corrected geometric mean (±SEM) percentages of absorption of ferrous sulfate alone and of ferrous sulfate in competition with iron *bis*-glycine chelate through the non-heme iron absorption pathway when both compounds were liberated at the intestinal level. The curves were significantly different ($P < 0.001$, repeated-measures ANOVA) and indicate that iron *bis*-glycine chelate competed with ferrous sulfate for the nonheme-iron absorption pathway when both compounds were liberated at the intestinal level. Absorption of the 0.5-mg dose of iron as ferrous sulfate or hemoglobin (ie, the reference dose) is defined as 100% absorption.

the values of iron absorption were 100.0%, 62.1%, 18.3%, and 11.4% for 0.5, 5, 50, and 100 mg Fe, respectively. The fitted dose-response curve of iron *bis*-glycine chelate absorption was significantly different from that of ferrous sulfate (repeated-measures ANOVA, $P < 0.001$), as shown in **Figure 3**. When the dose-response curves for the absorption of ferrous sulfate and iron *bis*-glycine chelate administered in gastric or enteric capsules were compared (Figures 1 and 3), a significant difference between gastric and enteric capsules was found only for the *bis*-glycine chelate (repeated-measures ANOVA, $P = 0.001$).

DISCUSSION

In our study, we demonstrated that iron *bis*-glycine chelate did not compete with heme iron for the heme-iron absorption pathway. If it had done so, we would have seen that the dose-response curves were the same for the absorption of hemoglobin and that of hemoglobin supplemented with iron *bis*-glycine chelate. The mild decrease in the dose-response curve for competition between iron *bis*-glycine chelate and hemoglobin may be attributed to the competition between a small fraction of nonheme iron dissociated from labeled hemoglobin and nonheme iron from iron *bis*-glycine chelate (Figure 2). The dose-response curves for absorption of ferrous sulfate alone and with iron *bis*-glycine chelate were similar (Figure 1), which shows that the iron from the *bis*-glycine chelate competed for the nonheme-iron absorption pathway. It is clear that a significant proportion of the chelated iron was released in the stomach, probably as a result of the effect of the low pH. Once released, iron probably became part of the nonheme-iron pool and was absorbed by the nonheme-iron absorption pathway.

With the purpose of avoiding the effect of the gastric environment on the dissociation of iron *bis*-glycine chelate, the compounds studied were contained in enteric capsules, which ensured that the compounds would be delivered to the duodenum without processing. The dose-response curves of ferrous sulfate and iron *bis*-glycine chelate show a similar trend, which again indicated that they competed via the nonheme-iron absorption pathway. However, the effect of iron *bis*-glycine chelate was more pronounced ($P < 0.001$). The most plausible explanation for this finding may be the different amounts of bioavailable iron offered to the enterocyte. In the environment of the duodenum, iron is probably protected by the glycine moiety, both chelating and isolating iron from intraluminal inhibitors, which results in a higher proportion of the iron being available to compete with ferrous sulfate absorption.

Iron *bis*-glycine chelate had better bioavailability than did ferrous sulfate when used in milk, corn flour, whole maize, and wheat flour. Olivares et al (6) showed that whole cow milk fortified with 15 mg iron *bis*-glycine chelate/L was absorbed at 11%, a level significantly higher than the 4% absorption seen with ferrous sulfate (17). Layrisse et al (8) recently showed that iron from the amino acid chelate was absorbed twice as well as iron from ferrous sulfate in a breakfast meal based on corn flour containing large amounts of phytates and polyphenols. They noted that the increase in absorption of iron *bis*-glycine chelate was probably due to the chemical structure of this compound, which partially prevents iron-phytate interactions (8). Similarly, Bovell-Benjamin et al (7) found that iron *bis*-glycine chelate in iron-fortified whole maize had better bioavailability than did ferrous sulfate. In the studies done by Layrisse et al (8) and Bovell-Benjamin et al (7), the iron *bis*-glycine chelate was more highly absorbed than was ferrous sulfate when these compounds were administered separately or together in the same meal. These results may suggest that the iron from the *bis*-glycine chelate is not exchanged with the iron from the nonheme-iron pool.

Our findings contradict these observations. We specifically designed our study to answer the question of whether iron from amino acid chelate competed for the nonheme-iron absorption pathway. Furthermore, in the studies mentioned, low doses of iron with a ratio of iron *bis*-glycine chelate to ferrous sulfate of 1:1 were administered. In contrast, in our study, this ratio ranged from 1:10 to 1:200. It is possible that, at low doses of iron and at a ratio of 1:1, competition between the 2 compounds cannot be observed. The reason for this is that, in spite of the greater availability of iron from the amino acid chelate, the quantity of available iron at these doses and ratio would not be sufficient to saturate the receptors and transporters of iron in the enterocyte. In our study, we observed a competition between the 2 compounds at higher doses and at ratios > 1.

In summary, our results show that the iron from iron *bis*-glycine chelate delivered at the level of the stomach or duodenum forms part of the nonheme-iron pool. The design of this study does not permit us to exclude the possibility that a minor fraction of iron *bis*-glycine chelate is absorbed through a pathway different from that of heme or nonheme iron. Further research is required to answer the question whether the competition for iron-absorption pathways between iron *bis*-glycine chelate and nonheme iron occurs at the cellular level.

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